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Nucleotide Sequence of a cDNA for a Water Channel Protein (Aquaporin) Homolog from *Atriplex canescens* (Pursh.) Nutt.

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A number of putative Aquaporins, water channel proteins related to the lens Major Intrinsic Protein (MIP; Gorin et al. 1984), have now been isolated (Chrispeels and Agre 1994). Various isoforms may be involved in mediating the passage of water across the membrane (Preston et al. 1992, Chasan 1992, Chrispeels and Agre 1994). Assembling a more comprehensive list of sequences may assist in identifying features which may then be correlated to physiological and biochemical activities. Most molecular work on plant stress has been carried out with glycophytes, however, halophytes may possess novel variations on established themes which are responsible for their success in extreme climates. Here, we report the cloning and sequencing of an *Atriplex* cDNA which is homologous to a pea MIP protein.

An *Atriplex canescens* (Saltbush) cDNA library was constructed using RNA from water deficit-stressed plants. This library was differentially screened and several clones of water deficit-inducible genes were isolated (Adair et al. 1992). Northern analysis confirmed the inducible nature of the corresponding genes, and a number of clones where the size of the cDNA approximated to that of the mRNA to which it hybridized were sequenced. One such clone, p8-2, encoded a protein which shows very strong homology to a turgor-responsive, Ion Channel Protein from pea (Guerrero et al. 1990). The homology between the *Atriplex* and pea proteins (68% identity, 83% similarity) extends throughout their length, 251 and 277 residues, respectively. The protein possesses six, spaced hydrophobic domains which may be transmembrane, a feature consistent with a model for the secondary structure of this protein (Guerrero et al. 1990, Yamaguchi-Shinozaki et al. 1992, Chrispeels and Agre 1994).

Table 1. Characteristics of a cDNA clone for a Water Channel Protein Homolog

Organism:
Atriplex canescens (Pursh.) Nutt.

Location in Genome:
Nuclear genome.

Chromosomal Location:
Not determined.

Gene Copy Number:
Not determined

Gene Product:
A homolog of a drought-inducible Ion Channel Protein from pea.

Function:
Not determined.

Clone Type:
SacI-XhoI cDNA fragment cloned into Bluescript vector and designated p8-2.

Source:
cDNA library was constructed in phage vector λZAP using polyadenylated RNA isolated from 3-year-old plant growing at a water potential of -0.9 MPa.

Isolation:
Library was differently screened using first-strand, radiolabelled cDNA derived from plants at water potential -0.4MPa (control) and from plants at -0.9MPa (water deficit). Clones exhibiting differential hybridization to the two probes were isolated, rescreened, and rescued as "phagemids." cDNA inserts were then used as probes in Northern analysis to confirm that water deficit caused steady state levels of the corresponding RNA to increase.

Method of Identification:
Homology; the putative polypeptide encoded by the *Atriplex* clone exhibits similarity to a drought-inducible Ion Channel homolog encoded by clone 7A from pea.

Sequencing Methods:
Homologous oligonucleotides were designed and used to prime dideoxy sequencing using Sequenase (United States Biochemicals). Both strands of the clone were completely sequenced in overlapping reactions.

Features of cDNA Sequence:
The cDNA is 1045 nucleotides in length and possesses a 5' untranslated region of 116 nucleotides followed by an open reading frame, beginging with ATG, which could encode a polypeptide of 282 amino acids. The clone includes a 3' untranslated region of 59 nucleotides and a polyA tail. The putative polypeptide has 68% identity, 83% similarity to a pea protein which is a member of the Major Intrinsic Protein (MIP) family of Ion Channel Proteins.

GC Content:
46.64%.

Structural Features of Protein:

The *Atriplex canescens* protein has a hydrophobicity plot similar to those observed in other members of the Major Intrinsic Protein (MIP) family. The structure of the hydrophobic and hydrophillic domains are consistent with the models of membrane spanning which have been suggested for MIP proteins.

Expression Profile:

The steady state RNA level rises in plants subjected to water deficit.

Antibodies:

Specific antibodies are not available.

Subcellular Localization of Protein Product:

To be determined.

Suborganellar Localization of Protein Product:

To be determined.

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The GenBank accession number for the sequence reported in this article is U18403

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